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# Xanthophyll-induced aggregation of LHCII as a switch between light-harvesting and energy dissipation systems<sup>☆</sup>

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## Abstract

The xanthophyll cycle pigments, violaxanthin and zeaxanthin, present outside the light-harvesting pigment–protein complexes of Photosystem II (LHCII) considerably enhance specific aggregation of proteins as revealed by analysis of the 77 K chlorophyll *a* fluorescence emission spectra. Analysis of the infrared absorption spectra in the Amide I region shows that the aggregation is associated with formation of intermolecular hydrogen bonding between the  $\alpha$  helices of neighboring complexes. The aggregation gives rise to new electronic energy levels, in the Soret region (530 nm) and corresponding to the Q spectral region (691 nm), as revealed by analysis of the resonance light scattering spectra. New electronic energy levels are interpreted in terms of exciton coupling of protein-bound photosynthetic pigments. The energy of the Q excitonic level of chlorophyll is not high enough to drive the light reactions of Photosystem II but better suited to transfer excitation energy to Photosystem I, which creates favourable energetic conditions for the state I–state II transition. The lack of fluorescence emission from this energy level, at physiological temperatures, is indicative of either very high thermal energy conversion rate or efficient excitation quenching by carotenoids. Chlorophyll *a* fluorescence was quenched up to 61% and 34% in the zeaxanthin- and violaxanthin-containing samples, respectively, as compared to pure LHCII. Enhanced aggregation of LHCII, observed in the presence of the xanthophyll cycle pigments, is discussed in terms of the switch between light-harvesting and energy dissipation systems.

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**Keywords:** Carotenoid; LHCII; Photoprotection; Protein aggregation; Xanthophyll cycle; Violaxanthin; Zeaxanthin

## 1. Introduction

Plants have developed several regulatory mechanisms, operating at all their organization levels, to optimize utilization of light energy driving the photosynthetic reactions. A movement of entire leaves [1], the photo-translocation of chloroplasts [2,3] and the phosphorylation-induced diffusion of the photosynthetic antenna complexes of Photosystem II (LHCII) within the thylakoid membranes [4,5] are the prominent examples of such a regulatory activity at the whole organism, cellular and subcellular level, respectively.

The regulation of excitation density in the photosynthetic apparatus is particularly important under overexcitation conditions, owing to the risk of light-induced generation of active oxygen species, leading to photo-degradation of plants. The xanthophyll cycle has been recognized as one of the most important regulatory mechanisms operating in plants and other photosynthesizing organisms at the molecular level under light stress conditions [6–8]. Under physiological conditions, the photosynthetic antenna complexes accumulate the xanthophyll pigment violaxanthin that becomes enzymatically de-epoxidized into zeaxanthin, upon excessive illumination. The back reaction, light-insensitive enzymatic epoxidation of zeaxanthin, completes turnover of the xanthophyll cycle. The fact that zeaxanthin is a carotenoid pigment that possesses longer conjugated double bond system, as compared to violaxanthin (11 versus 9 conjugated

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double bonds), implies lower localization of the lowest excited singlet state (S1), on the energy scale, with respect to the  $Q_y$  level of chlorophyll *a* and suggests the possibility of chlorophyll *a* excessive singlet excitation quenching by zeaxanthin but not by violaxanthin [9]. On the other hand, the recent determination of the energy levels of the xanthophyll cycle pigments shows that the difference between the energies of the S1 state of violaxanthin and zeaxanthin is very little and therefore essentially different chlorophyll excitation quenching efficiencies may not be expected [10]. Accordingly, no exceptional chlorophyll singlet excitation quenching has been observed in the antenna complexes isolated from the *lut* mutants of *Arabidopsis thaliana*, in which lutein has been replaced with zeaxanthin [11]. A very important aspect of the operation of the xanthophyll cycle in the thylakoid membranes is the direct presence of carotenoid pigments in the lipid phase of the thylakoid membrane and not only assembled into the pigment–protein complexes [12–15]. Moreover, under prolong light stress a pool of zeaxanthin in the thylakoid membrane increases considerably and exceeds the number of available violaxanthin binding sites in the antenna proteins, owing to the *de novo* synthesis of this xanthophyll pigment [16,17]. In this report we present the results of the experiments, which show that the xanthophyll cycle pigments, violaxanthin and zeaxanthin, present outside with respect to the LHCII complexes, enhance considerably aggregation of the pigment–proteins and that such an aggregation influences essentially the spectroscopic properties of photosynthetic pigments, giving rise to the exceptionally favorable conditions in terms of protection against over-excitation of Photosystem II. Formation of long-range LHCII aggregated structures, *in vitro* and *in vivo*, characterized by efficient thermal excitation energy dissipation has been reported in the literature [18,19] and is recognized to play a crucial function in photoprotection at the molecular level [20,21]. The structural basis of photoprotection in plants at the molecular level is a topic of very dynamic research [22–24] (see also [25] for an update). In general, the recent findings reveal two important aspects of photoprotection at the level of a photosynthetic light harvesting complex: a conformational transformation of the protein (an excitation quenching observed even without zeaxanthin [23]) and an excessive energy transfer from chlorophylls to zeaxanthin [24], proposed to be realized via the mechanism of zeaxanthin cation formation in the chlorophyll–carotenoid heterodimer [22]. It has been also shown that fast component of the excitation quenching in the antenna complexes network of Photosystem II, that develops within seconds (and therefore most probably independent of the xanthophyll cycle pigments) may be directly related to the Psbs protein, a member of the Lhc family associated *in vivo* with major antenna complexes [26]. The results of our study demonstrate an exceptional efficacy of the xanthophyll cycle pigments in promoting aggregation of LHCII, associated with appearance of new energy level of protein-bound chlorophylls, that can play physiological function in protection of the photosynthetic apparatus against overexcitation-induced damage.

## 2. Materials and methods

LHCII was separated from PSII particles prepared from *Zea mays* (cv. Hidosil) leaves as described previously [27]. The samples were finally purified using the UFC4 ODV 25 membranes and suspended in a solution containing 25 mM HEPES (pH 7.6), 0.06% n-dodecyl  $\beta$ -D-maltoside (DM) and protease inhibitors 1 mM benzamidine, 0.2 mM PMSF and 5 mM aminocaproic acid. The chlorophyll *a* to chlorophyll *b* ratio in the preparation was 1.3 and endogenous xanthophyll pigments: lutein, neoxanthin and violaxanthin were in the ratio as 2.0:0.6:0.2 per protein monomer, respectively. Violaxanthin was isolated from *Viola tricolor* blossoms and zeaxanthin from the fruits of *Lycium barbarum* and purified chromatographically as described previously [28,29]. Directly before the experiments xanthophylls were transferred to 0.25% DM in 10 mM HEPES buffer pH 7.6 and diluted up to 0.025% DM with the same buffer. Carotenoid solutions were centrifuged for 10 min at 20,000 $\times$ g in order to remove pigment aggregates and diluted up to the same concentration determined spectrophotometrically. LHCII was transferred to 0.025% DM prepared in 10 mM HEPES buffer pH 7.6. Samples were prepared by mixing 0.3 ml of LHCII suspension with 0.5 ml of the solution of violaxanthin or zeaxanthin, in a proportion one exogenous xanthophyll pigment per LHCII monomer, or pure buffer containing 0.025% DM in the case of the control. In order to achieve LHCII aggregation the samples were 10-fold diluted with the buffer and the suspension containing 0.0025% DM was subjected to 10 min centrifugation at acceleration 20,000 $\times$ g at 5 °C. The pellet containing aggregated LHCII was resuspended in 1.4 ml 10 mM HEPES buffer, pH 7.6 containing 0.0025% DM. In order to carry Fourier-transform infrared absorption (FTIR) measurements samples were deposited to a ZnSe support by evaporation. The electronic absorption spectra of LHCII deposited to ZnSe, analyzed in the red spectral region, were very close to the absorption spectra of LHCII in the buffer containing 0.0025% DM, moreover the measurements were taken in argon atmosphere and therefore in our opinion the samples were intact and not subjected to oxidative conditions. FTIR, light absorption, and fluorescence measurements were carried out as described previously [30,31]. Resonance light scattering spectra were recorded in a synchronous mode of the Shimadzu 5001 PC spectrofluorometer with the excitation and emission slits set to 1.5 nm and corrected for the spectrum of the lamp. Experiments were conducted at 25 °C unless indicated. Relative fluorescence quantum yield of chlorophyll *a* in LHCII was calculated on the basis of integration of absolute emission spectra in the spectral range 600–900 nm (excitation wavelength 440 nm). The results of integrations were divided by the results of integration of the 1-minus-transmission spectra (corrected for the Rayleigh type light scattering), performed in the spectral range 438–442 nm. All experiments were repeated at least four times and all the spectral effects reported are reproducible.

## 3. Results and discussion

Aggregation of LHCII which remains largely in a trimeric form at a DM concentration of 0.025% [32,33], was induced in the present study by a 10-fold dilution of the suspension with detergent free buffer, followed by 10-min centrifugation at acceleration of 20,000 $\times$ g. The pellet containing aggregated LHCII was collected and resuspended in the same buffer containing 0.0025% DM. Despite the fact that initial concentration of LHCII was identical in the samples containing pure protein and supplemented with exogenous violaxanthin and zeaxanthin, the final concentrations of the aggregated LHCII samples were different, after such a treatment. This can be seen directly from the intensity of the electronic absorption spectra presented in Fig. 1. As can be seen, the light scattering component, that supports the spectra, is also different in all kind of samples (even after absorbance normalization, not shown). The differences in light scattering observed in the samples composed of pure LHCII and supplemented with additional xanthophyll pigments are indicative of different type

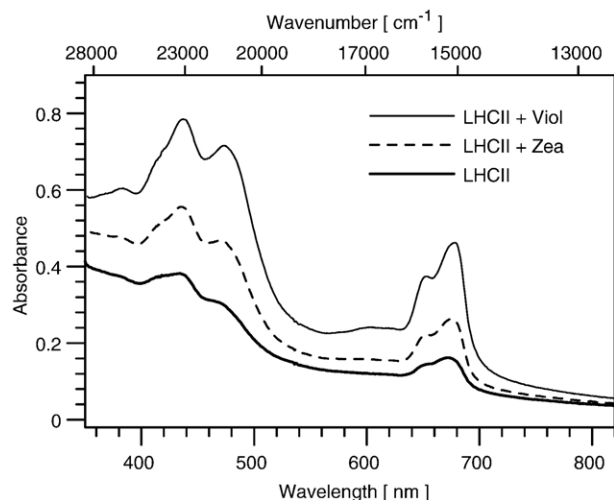


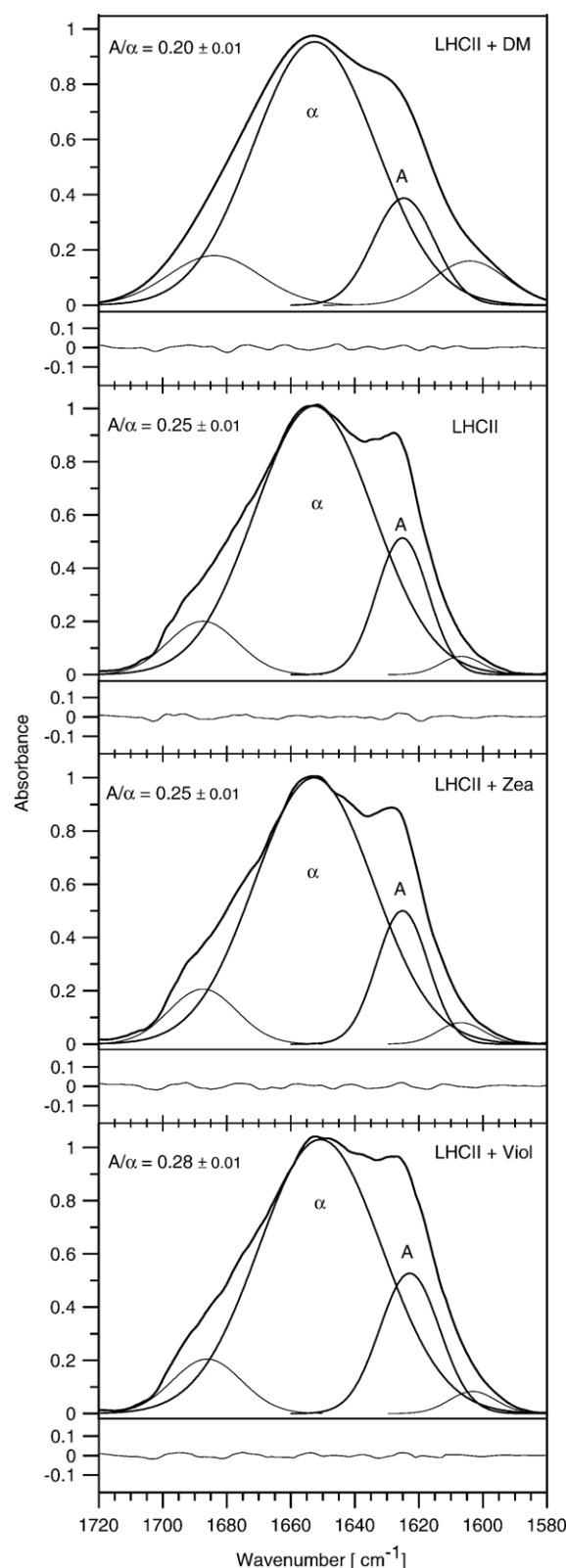
Fig. 1. Electronic absorption spectra of LHCII suspended in 0.0025% DM, pure and containing exogenous violaxanthin and zeaxanthin, indicated. See the text for more explanation.

of aggregated structures formed (in terms of scatter particle size and/or concentration) [30,34]. Much more efficient LHCII aggregation was obtained in the samples containing exogenous carotenoids. The electronic absorption spectra of the xanthophyll-supplemented LHCII samples display also some broadening of both the Soret and the Q bands (that can be seen from the difference of the spectra normalized at the  $Q_y$  maximum, not shown). Such a broadening is typical of aggregated LHCII [34].

Fig. 2 presents the IR absorption spectra, in the Amide I region (corresponding mostly to the peptide bond carbonyl group vibrations) of aggregated LHCII deposited from the detergent free buffer. Aggregated state of LHCII has an influence on the shape of the spectrum. The protein aggregation process is demonstrated by the presence of the absorption band centered at  $1625\text{ cm}^{-1}$ , accompanying the main band centered at  $1651\text{ cm}^{-1}$  that represents a protein  $\alpha$ -helical structure [35]. The origin of this “aggregation” band is associated with the formation of hydrogen bonds between the carbonyl groups and the amino groups of residues of neighboring  $\alpha$  helices and formally such a spectral band corresponds to the antiparallel  $\beta$  structure [36]. The new hydrogen bonds are generally not formed in expense of the hydrogen bonds involved in stabilization of the  $\alpha$  helices, since the proportion of the spectral component bands attributed to the helices and the turns and loops ( $1684\text{ cm}^{-1}$ ) are changed only slightly upon the protein aggregation. Considering the orientation and localization of the  $\alpha$  helices that constitute the

Fig. 2. Infrared absorption spectra of LHCII in the Amide I region recorded from the pure LHCII sample deposited from the HEPES buffer containing 0.0025% DM (the molar ratio LHCII:DM was 2.9), LHCII deposited for the pure buffer and the sample composed of LHCII and exogenous zeaxanthin and violaxanthin, deposited from the pure buffer, indicated. The main Gaussian components represent the  $\alpha$ -helical structure (centre at  $1651\text{ cm}^{-1}$ ) and the protein aggregated structure ( $1625\text{ cm}^{-1}$ ). The ratio of the area beneath those spectral components, corresponding to the aggregated and the helical forms ( $A/\alpha \pm \text{S.D.}$ ) is shown in each panel. The original spectra, the deconvolution components and the residuals of the fits are presented.

LHCII monomer, it is highly probable that exclusively the helix C, that is roughly parallel to the axis of the complex (normal to the thylakoid membrane) and located in the peripheral region of the molecule of LHCII and also in the peripheral region of the trimer [37,38], can contribute to the intermolecular hydrogen



bonding observed. It is therefore very likely that the aggregation of LHCII, manifested by the increased intensity of the  $1625\text{ cm}^{-1}$  band, is actually a dimerization of monomers or rather association of trimers into the structures in which the component trimers bind to each other via hydrogen bonding between the  $\alpha$  helices C of individual monomers. Such an interaction of trimers would explain the tendency of LHCII to form regular supra-structures of LHCII trimers observed in the isolated photosynthetic membranes [39] and in the Langmuir–Blodgett films [40].

As it might be expected, the aggregated LHCII sample deposited from the detergent free buffer, represents slightly higher aggregation level, measured as a ratio of areas beneath the spectral components representing the aggregate and the  $\alpha$ -helical structures ( $A/\alpha$ ) than the sample deposited from the protein suspension containing 0.0025% DM (LHCII:DM molar ratio 2.9). There were no spectral differences in the Amide I band, between the samples deposited by evaporation, composed of pure LHCII and containing one exogenous xanthophyll pigment per monomer (Fig. 2). Such a result indicates that in all cases the same type of aggregated structures is formed during the deposition.

Fig. 3 presents the resonance light scattering (RLS) spectra of aggregated LHCII in 0.0025% DM. The spectrum of pure LHCII displays two distinct maxima, at 526 nm (center of the band at 530 nm), in the Soret region, and at 691 nm, in the long-wavelength spectral region. The position of this long-wavelength band is exactly the same as in the case of light scattering by the LHCII aggregates formed at high salt concentration (30 mM  $\text{Mg}^{2+}$  plus 200 mM  $\text{K}^+$ ) [34]. The RLS spectra of the LHCII samples containing exogenous xanthophylls present different band shape. This effect can be caused by contribution from the carotenoid aggregates remaining in the samples. Despite those changes in the RLS spectra, the positions of both the short-wavelength and the long-wavelength maxima were very close ( $527 \pm 2$  nm and  $691 \pm 1$  nm). This shows that carotenoids promote the LHCII

aggregation process without essentially changing the photo-physical properties of the aggregated structures. Such a finding goes along with the results of the analysis based on the FTIR measurements presented above. According to the theory, resonance light scattering maxima are preferentially observed in the spectral regions corresponding to the “bottom” of an excitonic bands [41] and therefore the scattering bands observed at 530 nm and at 691 nm, in the case of the LHCII aggregated structures, can be attributed to the Soret band of the protein-bound chlorophyll and carotenoid pigments and to the Q spectral band of chlorophylls respectively, excitonically-shifted towards lower energies [41,42]. The resonance light scattering band in the short-wavelength region observed in this work in the 530 nm region corresponds directly to the band observed in the absorption spectra of intact leaves [43] and isolated LHCII [44], attributed to light scattering and correlated to the so-called high energy state excitation quenching [45]. A spectral band at this particular position has been also observed in the samples composed of zeaxanthin in the aggregated state and assigned to the low-energy excitonic level [46]. This suggests that LHCII-bound xanthophyll pigments can contribute to the 530 nm band, under conditions of aggregated pigment–protein complex.

Differences in the aggregation level in LHCII samples can be analyzed quantitatively on the basis of the 77 K chlorophyll *a* fluorescence emission spectra [31,47]. The low-temperature emission spectra display two prominent bands, one centered at 685 nm, characteristic of trimeric LHCII, and the second band, centered at 701 nm and characteristic of LHCII in the aggregated form [31,47]. As can be seen from Fig. 4, the aggregation level, represented by the ratio of the areas beneath the Gaussian components at 701 nm and 685 nm ( $A/T$ ) is evidently higher in the case of the samples containing exogenous zeaxanthin ( $1.23 \pm 0.11$ ) and exogenous violaxanthin ( $3.10 \pm 0.07$ ) as compared to the samples containing pure LHCII ( $0.62 \pm 0.06$ , average from six experiments  $\pm$  S.D.). Interestingly, the most efficient protein aggregation has been observed in the samples containing the xanthophyll pigment violaxanthin. Exactly the same strong violaxanthin effect has been observed in the carotenoid-modified monomolecular layers formed at the argon–water interface and in Langmuir–Blodgett films of LHCII [31]. The zeaxanthin-enhanced LHCII aggregation has been reported for the first time by Ruban et al. [48] but the authors reported that violaxanthin inhibited the aggregation of LHCII (induced by the local anaesthetic dibucaine). Effects of xanthophyll pigments in promoting aggregation of LHCII should depend, in principle, on both the xanthophyll–protein and xanthophyll–xanthophyll interactions. Owing to the fact that both mechanisms are competitive, the overall effect will depend on actual xanthophyll aggregation status and therefore one can expect different efficacy of the carotenoid-induced LHCII aggregation in dependence of initial exogenous xanthophyll concentration and individual xanthophyll aggregation threshold in a medium (such as detergent solution, monolayer film, lipid bilayer, etc.). Regardless differences observed, the results of the experiments presented in this work

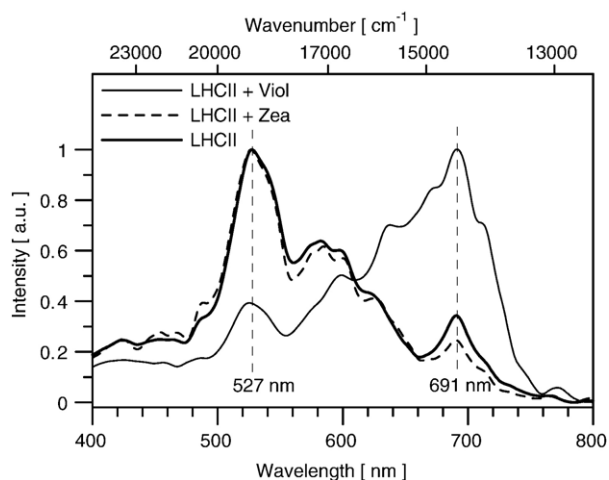


Fig. 3. Resonance light scattering spectra recorded from the pure LHCII sample in HEPES buffer containing 0.0025% DM and from the samples aggregated in the presence of exogenous violaxanthin and zeaxanthin, indicated. The positions of the maxima in the Soret band region and in the Q spectral region are indicated.



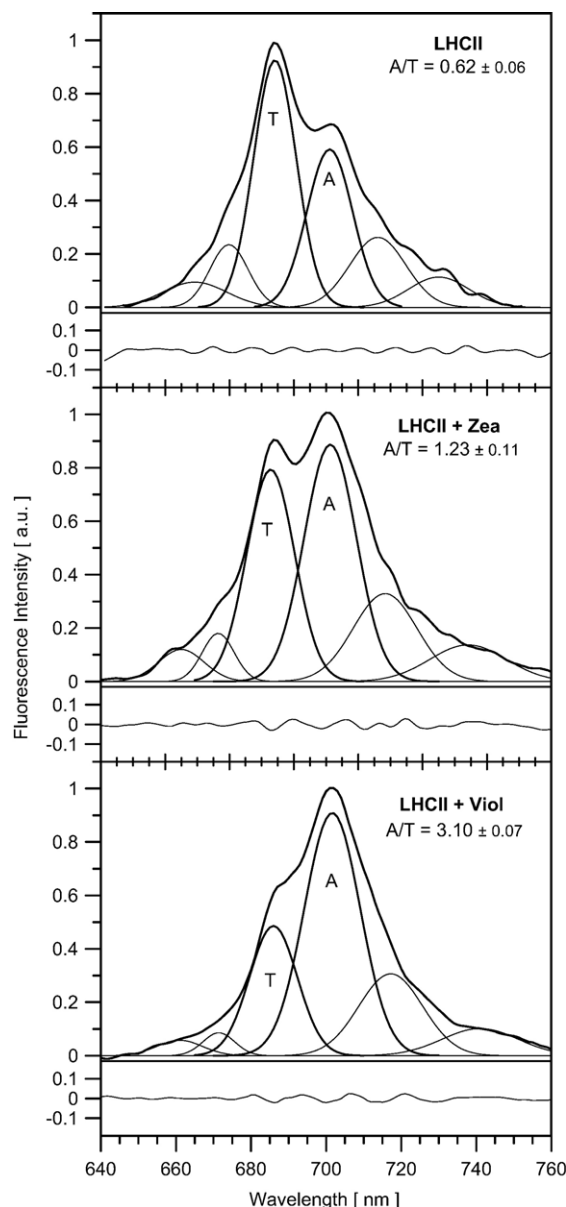


Fig. 4. 77 K chlorophyll *a* fluorescence emission spectra recorded from LHCII samples in HEPES buffer containing 0.0025% DM, pure LHCII and containing exogenous violaxanthin and zeaxanthin, indicated. Excitation at 440 nm, excitation and emission bandwidths 3 and 1.5 nm, respectively. The Gaussian deconvolution components and residuals are presented along with the original spectra. In each panel is presented also the ratio of the areas below the spectral components at 701 nm and 685 nm, corresponding to the aggregated state (A) and the trimeric state (T) of LHCII respectively (A/T), as a measure of the aggregation process (average from 5 measurements  $\pm$  S.D.). The full width at half-height of the principal components at 701 nm and 685 nm were in the range 14–16 nm.

show that both xanthophyll pigments violaxanthin and zeaxanthin enhance effectively aggregation of LHCII.

One of the possible explanations of such a strong effect of xanthophyll pigments in promoting the LHCII aggregation can be based upon stabilization of the protein assemblies formed by intermolecular hydrogen bonding between the  $\alpha$  helices. Detailed molecular model of such a stabilization is beyond the scope of this work but we can speculate that van der Waals

interactions of exogenous xanthophylls with neoxanthin (located close to the helix C), on the one hand, and with the xanthophyll remaining in the violaxanthin binding site, on the other hand, may be involved in such a stabilization. It also may not be excluded that one xanthophyll pigment adopts appropriate steric conformation and binds simultaneously to both the violaxanthin- and the neoxanthin-binding pockets of the protein. Regarding the type of aggregates formed in the present experiments we think that they correspond to the LHCII aggregates studied by Garab et al. [49,50], except that our samples were enriched with exogenous xanthophyll cycle pigments.

The fact that the long-wavelength 77 K fluorescence emission band centered at 701 nm appears exclusively in the aggregated LHCII samples, characterized also by the excitonic band at 691 nm, suggests that just this band is a spectral origin of the irradiative de-excitation observed (with a Stokes shift of 10 nm). Fig. 5 presents the 77 K fluorescence excitation spectra of LHCII recorded with the emission monochromator set to 680 nm and 700 nm. Appearance of the spectral component in the long-wavelength edge of the excitation band, which matches the excitonic band visible at 530 nm in the RLS spectrum (Fig. 3) supports strongly such an interpretation. The bathochromic spectral shift of the  $Q_y$  band observed, from 678 nm to 691 nm ( $\Delta\nu \approx 277 \text{ cm}^{-1}$ ) is relatively large and reflects strong excitonic interactions between chromophores involved ( $\beta$ ). In the case of linear aggregates a shift of  $\Delta\nu = 2\beta$  is to be expected [41]. Assuming formation of such a kind of aggregates one arrives to the exciton coupling energies as high as  $\beta = 138.5 \text{ cm}^{-1}$  between the chlorophyll molecules in the aggregated LHCII. The parameter  $\beta$ , found in the present work for aggregated LHCII, is not as high as in the case of the aggregated forms of chlorophyll *a* in organic solvents ( $400 \text{ cm}^{-1}$  [41]) but comparable with the dipole–dipole interaction energies between chlorophyll molecules embedded in the LHCII protein (e.g.

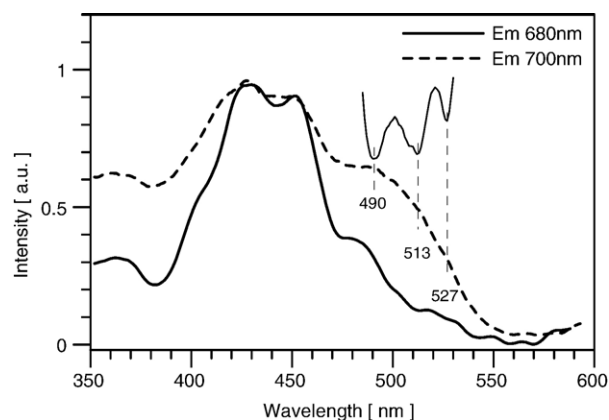


Fig. 5. 77 K chlorophyll *a* fluorescence excitation spectra of exogenous violaxanthin-containing LHCII suspension in the HEPES buffer containing 0.0025% DM. Emission wavelength 680 nm (solid line) and 700 nm (dashed line). Excitation and emission bandwidths 1.5 and 3 nm, respectively. The second derivative spectrum in the long wavelength region of the spectrum recorded with emission at 700 nm is also presented. The minima of the derivative spectrum corresponding to the most red-shifted excitation band components indicated.

144.9  $\text{cm}^{-1}$  Chla 611–612 or 123.4  $\text{cm}^{-1}$  Chlb 606–Chla 604, supplementary information to Ref. [38]). This comparison suggests that the process of LHCII aggregation observed does not change the locations of individual pigments but rather is limited to increase of a number of excitonically coupled chromophores. At this stage of research we may only speculate that the chlorophyll pairs excitonically coupled and close to the helix C can potentially be also involved in the excitonic interactions between the trimers in aggregated LHCII. It is therefore possible that the Chlb 606–Chla 604 pair of one LHCII, interacts with the pair Chla 603–Chlb 609 of the other LHCII complex and vice versa.

Fig. 6 presents the chlorophyll *a* fluorescence emission spectrum recorded from the aggregated LHCII sample at room temperature. As can be seen, fluorescence spectrum is typical of LHCII in room temperature but differs substantially from that one recorded at 77 K, in particular the distinct emission band centered at 701 nm and attributed to the chlorophyll *a*  $Q_y$  excitonic band is missing. One of the possible explanations of this effect could be very high energy thermal conversion rate from this level. Another possibility could be a singlet–singlet excitation energy transfer from this lowest excitonic band of chlorophyll *a*, in the aggregated LHCII, to the protein-bound xanthophyll pigments, assuming that such an energy transfer is less effective upon protein transfer into the liquid nitrogen.

The comparison of appropriate energy levels, potentially involved in such a process, is presented in Fig. 7. Both the short range energy–electron exchange and the long range resonance energy transfer mechanisms depend on the overlap of the emission band of the donor and the absorption band of the acceptor. In this respect, the appearance of an additional, low-energy excitonic band of chlorophyll *a* ( $Q_y^{\text{EB}}$ ), in the region of the S1 energy levels of the LHCII-bound xanthophyll pigments, will improve conditions for an effective chlorophyll *a* excitation energy quenching [24]. It is worth mentioning that the relative chlorophyll *a* fluorescence quantum yield in LHCII, determined on the basis of fluorescence lifetime measurements, was distinctly decreased upon the incorporation of exogenous xanthophylls into the system [51]. It was found in the present

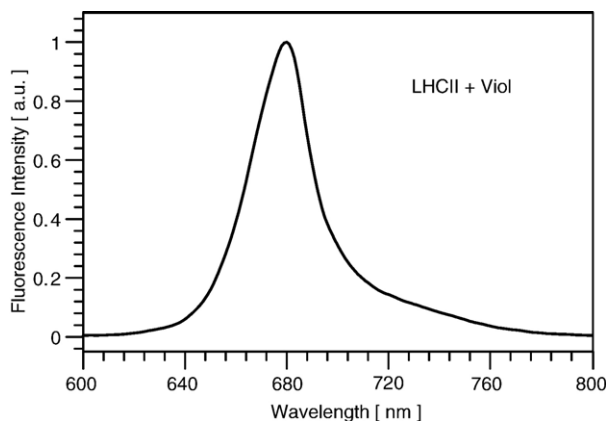


Fig. 6. Chlorophyll *a* fluorescence emission spectrum recorded at 25 °C from the exogenous violaxanthin-supplemented LHCII suspension in the HEPES buffer containing 0.0025% DM. Excitation at 440 nm. Excitation and emission bandwidths 3 and 1.5 nm, respectively.

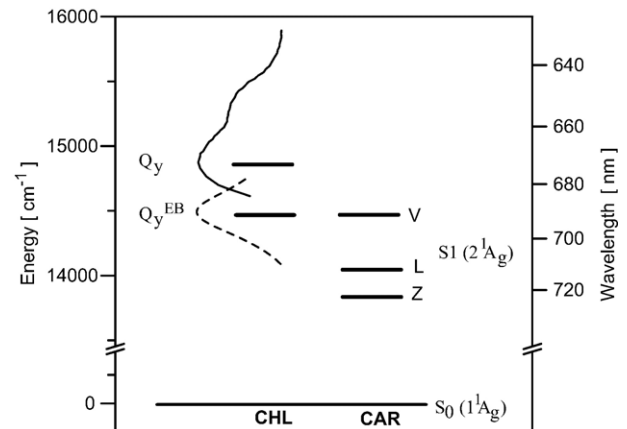


Fig. 7. Energy level diagram. The localization of the  $Q_y$  level and the excitonic band level  $Q_y^{\text{EB}}$  of chlorophyll *a* (CHL) is based on the absorption spectrum of LHCII (Fig. 1) and the resonance light scattering spectrum (Fig. 3), respectively (stacked on the energy level diagram) and the positions of the S1 energy level ( $2^1A_g$ ) of carotenoids (CAR): violaxanthin (V,  $13,700 \pm 300 \text{ cm}^{-1}$ ), lutein (L,  $14,050 \pm 300 \text{ cm}^{-1}$ ) and zeaxanthin (Z,  $13,850 \pm 200 \text{ cm}^{-1}$ ) is based on the literature [10]. The energies of the S1 state of xanthophylls, determined on the basis of the S1→S2 transition for the pigments embedded in the LHCII environment [52] were selected for a display.

work that the chlorophyll *a* fluorescence quantum yield, determined on the basis of integration of the absolute fluorescence emission spectra recorded at room temperature, in the samples containing pure LHCII, LHCII with exogenous zeaxanthin and LHCII with exogenous violaxanthin were in a ratio as  $1:0.61 \pm 0.01:0.34 \pm 0.03$  respectively (average from four experiments  $\pm$  S.D.). Such a result confirms ability of xanthophyll pigments to promote excitation quenching in LHCII that accompanies antenna protein aggregation. Fig. 8 presents comparison of the absorption spectrum of lutein ( $S_0 \rightarrow S_2$  transition), shifted towards lower energies in order to

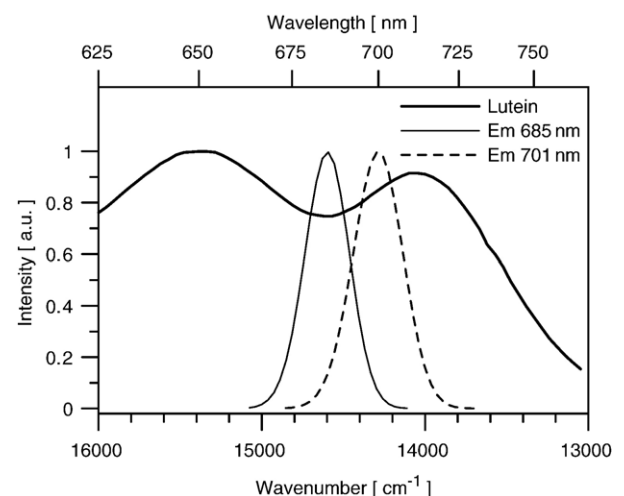


Fig. 8. Comparison of the absorption spectrum of lutein recorded in ethanol ( $S_0 \rightarrow S_2$  transition), shifted towards lower energies in order to position the maximum corresponding to the 0–0 vibrational transition to the energy determined for the S1 state (see Fig. 7), with the chlorophyll *a* fluorescence emission band components in LHCII in the trimeric and aggregated forms, represented by the Gaussian components, the same as in Fig. 4, indicated. The spectra were normalized in the maximum.

position the maximum corresponding to the 0–0 vibrational transition at the energy determined for the S1 state, with the chlorophyll *a* fluorescence emission band components in LHCII, in the trimeric and aggregated forms. The S1 absorption band of carotenoids cannot be measured directly owing to the fact that the S0→S1 transition is not allowed for the symmetry reasons. As mentioned above, an overlap of the emission spectrum of chlorophyll *a* with the absorption spectrum of a carotenoid is necessary for efficient chlorophyll excitation quenching. Both the chlorophyll *a* emission bands, corresponding to the trimeric and aggregated LHCII are relatively sharp as compared to the carotenoid absorption band and therefore pronounced differences in the spectral overlap integrals may not be expected. Indeed, the spectral overlap calculated for violaxanthin and zeaxanthin was very close to each other, indicating that appearance of the new excitonic band of chlorophyll, as a result of LHCII aggregation, would not change essentially excitation energy transfer to those xanthophylls. On the other hand, the spectral overlap of the band simulated the S1 absorption of lutein was higher by 23% in the case of the emission band of aggregated LHCII (701 nm) as compared to the band corresponding to trimeric LHCII (685 nm). It is therefore possible that LHCII aggregation provides favorable conditions for chlorophyll excitation quenching by protein-bound lutein.

Aggregation of LHCII has been recognized as a major protective mechanism against overexcitation, since the excitation quenching, manifested by the pronounced decrease in the chlorophyll fluorescence lifetime, is associated with the protein oligomerization and crystallization [23]. Interestingly, the formation of crystal structure of LHCII, that results in a pronounced excitation quenching, is not associated with the interactions between the hydrophobic fragments of the trimers [23,38], as in the case of the aggregated structures stabilized by hydrogen bonds between  $\alpha$  helices, examined in this work. This shows that not a single protein structural transformation may lead to the same effect of the singlet excitation quenching.

The spectroscopic data presented in this report show clearly that both the xanthophyll cycle pigments, zeaxanthin and violaxanthin, present outside the photosynthetic antenna complexes of Photosystem II, promote formation of aggregated protein structures, stabilized by intermolecular hydrogen bonding. The LHCII aggregation is associated with excitonic interactions between chlorophyll pigments. These interactions give rise to the new, low-energy electronic level in the region of the lowest singlet excited level of the xanthophylls. Aggregation of LHCII is also associated with decrease of the extinction coefficients and with light scattering that competes for light quanta with absorption process and therefore decreases the absorption cross-section. The new excitonic band energy level can facilitate also energetic coupling to Photosystem I and energy balance between photosystems owing to the so-called state I–state II transition [4,5]. The onset of all of these processes is triggered by the LHCII aggregation promoted by the xanthophyll pigments and therefore this mechanism can be considered as a switch between light-harvesting and energy dissipation systems.

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